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PROCESS FOR THE IDENTIFICATION AND ISOLATION OF INSULIN PRODUCING BETA CELL DIFFERENTIATING FACTOR FROM THE PERIVITALLINE FLUID OF THE FERTILISED EGGS OF HORSESHOE CRAB

Field of the invention

This invention relates to the identification and characterization of cell proliferating factor in the peri-vitalline fluid of the fertilized eggs of the Indian horseshoe crab.

Background of the invention

The regeneration of pancreatic β-cells has become an important factor for the purpose in curing *Diabetes mellitus*. The concept that β-cell mass is static has changed and now we know that β-cells are dynamic and to certain extent can compensate for the loss and retain their ability to respond to changes in blood glucose by their ability to maintain glucose homeostasis. These studies are based on the experimental animal models. In continuation with the same efforts have been made to search for the factors that lead to direct differentiation of β-cells from precursor cells and those responsible for proliferation of existing β-cells in the residual β-cell mass in the diabetic individual. The aims of these efforts are to find out novel molecules to control and cure of diabetes by natural products.

Rat AR42J is derived from a chemically induced pancreatic tumor having exocrine origin and has the feature of pluri-potency of the common precursor cell of the pancreas. It has been reported that amylase secreting AR42J cells convert themselves into insulin secreting cells in the presence of hepatocyte growth factor and or in the presence of betacellulin and activin A. Reference may be made to a publication wherein rat AR42J cells were derived from a chemically induced pancreatic tumor having exocrine origin and have the feature of pluri-potency of the common precursor cell of the pancreas (Mashima H, Ohnishi H, Wakabayashi K, Mine T., Miyagawa J, Hanahusa Ta, Seno M, Yamada H, Kojima I, Betacellulin and activin A coordinately convert amyase-secreting pancreatic AR42J cells into insulin-secreting cells, *J Clin Invest* 97:1647-1654, 1996; Mashima H, Yamada S, Tajima T., Seno M, Yamada H, Takeda J and Kojima I. Genes Expressed During the Differentiating of Pancreatic AR42J cells into insulin – secreting cells, *Diabetes*, 48, p 304-309, 1999).

Object of the invention

The main object of the invention to identify and isolate insulin producing beta cell differentiating factor from the perivitalline fluid of the fertilised eggs of Horseshoe crab.

It is a further object of the invention to achieve the differentiation pancreatic AR42J cells into insulin producing cells.

It is another object of the present invention to provide a new process for isolation of new growth promoting activity from the peri-vitalline fluid collected from the fertilized eggs of the horseshoe crab that facilitates the proliferation of AR42J cells.

It is a further object of the invention to achieve the fractionation of potential putative differentiating factor from perivitalline fluid of a Horseshoe crab.

Summary of the invention

Accordingly, the present invention provides a process for identification of insulin producing β-cells proliferating factor from the peri-vitalline fluid of fertilized eggs of horseshoe crab that facilitates the proliferation of AR42J cells from rat origin.

In one embodiment of the invention, the process comprises collecting peri-vitalline fluid from the fertilized eggs of the Indian horseshoe crab.

In another embodiment of the invention, the peri-vitalline fluid is collected asceptically, aliquated and stored at –20° C.

In a further embodiment of the invention, the AR42J cell line (rat ascinar cells) obtained from American Type Culture Collection (Rockville MD, USA) was grown in Dulbeco's modified minimum essential medium supplemented with 10% FCS.

In a further embodiment of the invention, the cells were maintained in NUNC six-well dishes (NUNCLON, Denmark).

In another embodiment of the invention, a sub culture was done every 4^{th} day of seeding of 1×10^5 cells and the cultures were incubated at 37° C.

In yet another embodiment of the invention, concentration dependent proliferation was observed in presence of peri-vitalline fluid.

Detailed description of the invention

In preliminary studies it was observed that peri-vitalline fluid collected from the fertilized eggs of the horseshoe crab has proliferative and differentiating activity in AR42J cell line of rat pancreas.

The aforesaid process requires absolute precaution for bacterial and fungal contamination at all above processing steps. All apparatus and reagents must therefore, be pyrogen free. The novelty of the present invention is in identification of a new growth promoting activity from the peri-vitalline fluid collected from the fertilized eggs of the horseshoe crab that facilitates the proliferation of AR42J cells of rat origin.

Methodology

1. Collection of per-vitalline fluid from the fertilized eggs of the horseshoe crab:

Fertilized eggs of the horseshoe crab were collected from the nests located on the sandy beach at Balramgari (Orissa). The fertilized eggs were transferred in filtered seawater and incubated at a constant in artificial incubators. As soon as the eggs became transparent, showing the movement of trilobite larvae, the perivitalline fluid was collected aseptically, aliquated and stored at -20^0C.

2. Cell Culture:

AR42J cell line (rat ascinar cells) obtained from American Type Culture Collection (Rockville MD, USA) was grown in Dulbeco's modified minimum essential medium supplemented with 10% FOS. The cells were maintained in NUNC six-well dishes (NUNCLON, Denmark). Sub culture was done every fourth day of seeding of $1x10^5$ cells and the cultures were incubated at 37^0C in 5% CO_2 atmosphere. AR42J cells were grown in presence of perivitalline fluid and 3H-thymidine for 72 hours at 37^0C. Concentration dependent proliferation was observed (Table 1). Studies were being carried out to see mRNA expression of insulin in AR42J in presence of perivitalline fluid.

Example

Fertilized eggs of the horseshoe crab were collected from the nests located on the sandy beach at Balramgari (Orissa). The fertilized eggs were transferred in filtered seawater and incubated at a constant in artificial incubators. As soon as the eggs became transparent, showing the movement of trilobite larvae, the peri-vitalline fluid was collected asceptically, aliquated and stored at -20° C.

AR42J cell line (rat ascinar cells) obtained from American Type Culture Collection (Rockville MD, USA) was grown in Dulbeco's modified minimum essential medium supplemented with 10% FCS. These cells were maintained in NUNC six-well dishes (NUNCLON, Denmark). Sub culture was done every 4^{th} day of seeding of 1×10^5 cells and the cultures were incubated at 37° C in 5% Co_2 atmosphere. AR42J cells were grown in presence of peri-vitalline fluid and proliferative activity of peri-vitalline fluid was observed which was found to be concentration dependent (Table 1). Peri-vitalline fluid showed significant proliferation of AR42J cell lines which is reported for the first time.

Table 1: Percent increase in cell population in presence of peri-vitalline fluid.

Per-vitalline (µg/ml)	3H-counts/min	% increase
0.0	1678	-
1.0	2377	041
2.5	3115	085
5.0	8662	416
10.0	13083	680

Advantage: Identification of the new growth factor will be useful for cell proliferation.